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Regulation effects of water and nitrogen on yield, water, and nitrogen use efficiency of wolfberry

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Abstract: Wolfberry (*Lycium barbarum* L.) is important for health care and ecological protection. However, it faces problems of low productivity and resource utilization during planting. Exploring reasonable models for water and nitrogen management is important for solving these problems. Based on field trials in 2021 and 2022, this study analyzed the effects of controlling soil water and nitrogen application levels on wolfberry height, stem diameter, crown width, yield, and water (WUE) and nitrogen use efficiency (NUE). The upper and lower limits of soil water were controlled by the percentage of soil water content to field water capacity (θ_f), and four water levels, i.e., adequate irrigation (W0, 75%–85% θ_f), mild water deficit (W1, 65%–75% θ_f), moderate water deficit (W2, 55%–65% θ_f), and severe water deficit (W3, 45%–55% θ_f) were used, and three nitrogen application levels, i.e., no nitrogen (N0, 0 kg/hm^2), low nitrogen (N1, 150 kg/hm^2), medium nitrogen (N2, 300 kg/hm^2), and high nitrogen (N3, 450 kg/hm^2) were implied. The results showed that irrigation and nitrogen application significantly affected plant height, stem diameter, and crown width of wolfberry at different growth stages (P<0.01), and their maximum values were observed in W1N2, W0N2, and W1N3 treatments. Dry weight per plant and yield of wolfberry first increased and then decreased with increasing nitrogen application under the same water treatment. Dry weight per hundred grains and dry weight percentage increased with increasing nitrogen application under W0 treatment. However, under other water treatments, the values first increased and then decreased with increasing nitrogen application. Yield and its component of wolfberry first increased and then decreased as water deficit increased under the same nitrogen treatment. Irrigation water use efficiency (IWUE, 8.46 kg/(hm^2•mm)), WUE (6.83 kg/(hm^2•mm)), partial factor productivity of nitrogen (PFPN, 2.56 kg/kg), and NUE (14.29 kg/kg) reached their highest values in W2N2, W1N2, W1N2, and W1N1 treatments. Results of principal component analysis (PCA) showed that yield, WUE, and NUE were better in W1N2 treatment, making it a suitable water and nitrogen management mode for the irrigation area of the Yellow River in the Gansu Province, China and similar planting areas.

Keywords: water deficit; growth characteristics; yield; water and nitrogen use efficiency; principal component analysis

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1 Introduction

Wolfberry (*Lycium barbarum*) is a perennial deciduous shrub belonging to Solanaceae family (Yao et al., 2018). It is a saline-alkali resistant, barren resistant, and drought resistant plant

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(Wozniak et al., 2012) and has many biological activities, such as anti-aging, nerve protection, metabolism promotion, and immune regulation (Khoo et al., 2017; Muatasim et al., 2018). It is of great significance to improve saline-alkali land, wind prevention, sand fixing, and promoting human health (Kulczyński and Gramza-Michałowska, 2016). In recent years, wolfberry industry has experienced rapid development. By 2020, the planting area and output of Chinese wolfberry will reach more than 9.33×10^4 hm^2 and 4.00×10^5 t, respectively, accounting for more than 95% of the global total (Huang and Zhang, 2021). However, wolfberry is mainly cultivated in northwestern China, where the soil is poor and water resources are limited (Amagase and Farnsworth, 2011). For a long time, planting Chinese wolfberry consumed high amounts of water and fertilizer, resulting in a series of problems, such as low marginal yield of wolfberry, low water, and fertilizer utilization efficiency, large greenhouse gas emission, and declining soil quality (Zhang et al., 2015a). Therefore, exploring reasonable water and nitrogen management mode is important to improve wolfberry productivity and its sustainable development.

Water is an important resource for agricultural production and directly affects crop growth, development, and production (Lin et al., 2019; Ma et al., 2019). Appropriate water stress can stimulate the antioxidant defense mechanism and osmoregulation of plants, improve the stability of cell structure, and promote the accumulation of dry matter in crops (Liu et al., 2011). Excessive or insufficient irrigation can reduce the vitality of crop roots and slow down the absorption rate of soil nutrients for roots, which is not conducive to crop growth (Fu et al., 2021). Studies have found that a certain degree of water stress is beneficial to increase the plant height, stem diameter, crown width, and shoot growth of wolfberry (Qi et al., 2019; Jia et al., 2022). Yield, water use efficiency (WUE), and fruit quality of wolfberry increased with the increasing of irrigation amount, and showed a downward trend when the irrigation amount exceeded the threshold value (Xu and Zheng, 2009; Zhao et al., 2018). Fertilizer is another important resource in agricultural production, with nitrogen being particularly important (Lai et al., 2021). Reasonable application of nitrogen fertilizer can effectively improve the water absorption capacity of roots, coordinate the water and nitrogen metabolism of plants, promote photosynthesis, and delay the senescence of leaves (Tang et al., 2010). Studies have shown that appropriate nitrogen application can significantly increase the leaf area index and chlorophyll content of wolfberry, and promote the growth of fruit shape index (Zhang et al., 2018; He and Li, 2022). The yield components and WUE of wolfberry increased first and then decreased with the increases of nitrogen application (Lai et al., 2021; Ma et al., 2021). There is an interaction effect between water and nitrogen (Kong et al., 2012). The cooperative supply of water and nitrogen is the material basis of physiological metabolism for plants, and it is also an important guarantee for improving quality and efficiency during crop production (Stamatiadis et al., 2016; Li et al., 2019b). In India, Kumar et al. (2022) found that the cotton leaf area index, dry matter accumulation, growth rate, and relative growth rate were the highest with a nitrogen application of 225 kg/hm^2 and irrigation of 600 mm. In the North China Plain, Si et al. (2020) found that when the yield, WUE, and nitrogen use efficiency (NUE) of winter wheat showed a decreasing trend when water and nitrogen application reached the critical point. In Egypt, Badr et al. (2012) found that coupling nitrogen application with appropriate crop water demand could significantly increase NUE of potato. In Pakistan, Ahmad et al. (2019) found that WUE and economic benefits of maize were significantly improved when irrigation of 80%–100% field water capacity (θ_f) coupled 240 kg/hm^2 nitrogen application. In summary, the coupling control of water and nitrogen can achieve the effect of "promoting fertilizer with water and transferring water with fertilizer". However, the current researches on the coupling regulation of water and nitrogen mainly focused on cotton (Badr et al., 2012; Ali et al., 2019), wheat (Si et al., 2020; Li et al., 2022a), potato (Ahmad et al., 2019; Akkamis and Caliskan, 2023), corn (Liu et al., 2011; Qi and Hu, 2020), tomato (Wang et al., 2019a; Ghannem et al., 2021), and other crops (Djidonou et al., 2013; Gu et al., 2015; Lin et al., 2019). Studies on the production effect of economic forest of wolfberry mainly focused on the single factor regulation of water or nitrogen (Zhou et al., 2021). The study on the effect of cooperative supply of water and nitrogen on the production of wolfberry is helpful to stimulate the potential of wolfberry production. In addition, the determination of optimal irrigation and

nitrogen application combination of wolfberry is mostly based on single objective evaluation (Song et al., 2019; Wang et al., 2019b), and there are few studies on the evaluation of water and nitrogen regulation effects for wolfberry by integrating multiple objectives. Irrigation area in the Gansu Province is situated in the upper reaches of the Yellow River with abundant sunshine and a significant temperature difference between day and night, making it an ideal location for cultivating wolfberry because of its unique geographical advantages (Wang and Wang, 2018). In recent years, the development of the wolfberry industry in the irrigation area of the Yellow River in the Gansu Province has been rapid. Planting area and dried fruit output have both reached more than 45% of the national total, and it has become an important wolfberry planting base in China (Li et al., 2017). However, the shortage of water resources, extensive agricultural production, and low resource utilization rate in this area seriously restrict the development of local wolfberry industry (Wang and Xia, 2012; Qi et al., 2019). Therefore, the objectives of the research were as follows: (1) to analyze the effects of water and nitrogen regulation on growth, yield, WUE, and NUE of wolfberry; and (2) to comprehensively evaluation the water and nitrogen management mode that obtained the productivity improvement of wolfberry in study area and similar planting areas.

2 Materials and methods

2.1 Study area

The experiment was conducted at the Irrigation Experimental Station (37°23′N, 104°08′E) of the Jingtaichuan Electric Power Irrigation Water Resource Utilization Center in the Gansu Province of China from May to September 2021 and 2022. The experimental station is located in the irrigation area of the Yellow River in the Gansu Province, where wolfberries are widely cultivated. With an average altitude of 1562 m, the region has a temperate continental arid climate with low precipitation and high evaporation. The annual average precipitation, evaporation, temperature, sunshine duration, radiation, and frost-free period were 184 mm, 3029 mm, 8.6°C, 2652 h, 6.18×10^5 J/cm^2, and 191 d, respectively. The soil texture was sandy loam, the dry bulk desity was 1.61 g/cm^3, and the field water holding capacity was 24.10%. The soil organic matter, total nitrogen, total phosphorus, total potassium, available nitrogen, available phosphorus, and available potassium in the 0–60 cm soil layer were 1.32 g/kg, 1.62 g/kg, 1.32 g/kg, 34.03 g/kg, 74.51 mg/kg, 26.31 mg/kg, and 173.00 mg/kg, respectively, with a pH of 8.11. Meteorological data were obtained from a small weather station installed in the field. The distributions of precipitation and average temperature during the experiment are shown in Figure 1.

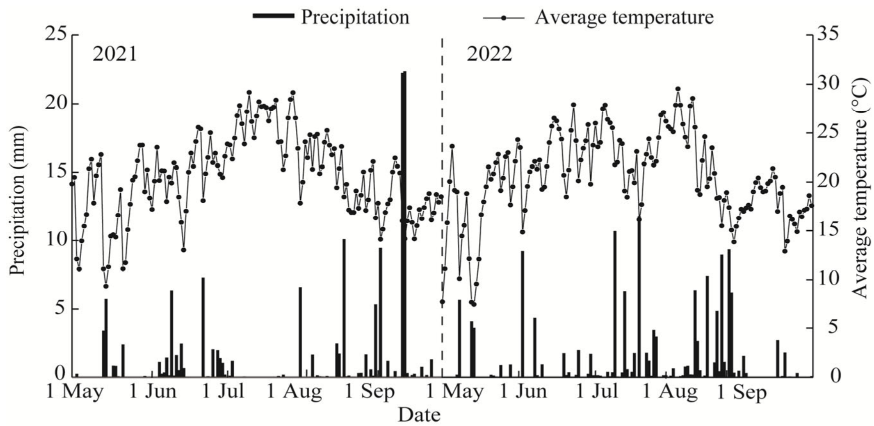

Fig. 1 Precipitation and average temperature at wolfberry growing stage from 2021 to 2022

2.2 Experimental design and soil sampling

The experiment adopted a randomized block design, and regarding local production practices and previous research results (Li et al., 2020a), two factors were established: water and nitrogen

application levels. The water levels were as follows: the depth of wet layer of the irrigation plan was 60 cm, and the upper and lower limits of soil water were controlled by the percentage of soil water content to field water capacity (θ_f), adequate irrigation (W0, 75%–85% θ_f), mild water deficit (W1, 65%–75% θ_f), moderate water deficit (W2, 55%–65% θ_f), and severe water deficit (W3, 45%–55% θ_f). Nitrogen application levels (pure nitrogen, urea, and 46% nitrogen content) were as follows: no nitrogen (N0, 0 kg/hm^2), low nitrogen application (N1, 150 kg/hm^2), medium nitrogen application (N2, 300 kg/hm^2), and high nitrogen application (N3, 450 kg/hm^2). Sixteen treatments were performed in three replicates. Nitrogen fertilizer was applied at a ratio of 6:2:2 at the vegetative growth, full flowering, and summer full fruit stages. Phosphate fertilizer ($Ca(H_2PO_4)_2$, 12% phosphorus content) and potassium fertilizer (KCl, 60% potassium content) were applied at 130 kg/hm^2 once a year at the germination stage. Wolfberry (Ningqi No. 5) was planted in early April 2021 with a plant row spacing of 1.5 m×3.0 m and five rows of five trees in each plot (76.5 m^2, 10.2 m×7.5 m), totaling 20 trees (Fig. 2). The experiment integrated drip irrigation with water and fertilizer. Irrigation pipes were laid in each district, and valves and water meters were installed to control the amount of irrigation. Drip irrigation was applied at a distance of 15 cm from the wolfberry trees at a flow rate of 2 L/h. Other field management practices and pest control were consistent with local farming practices.

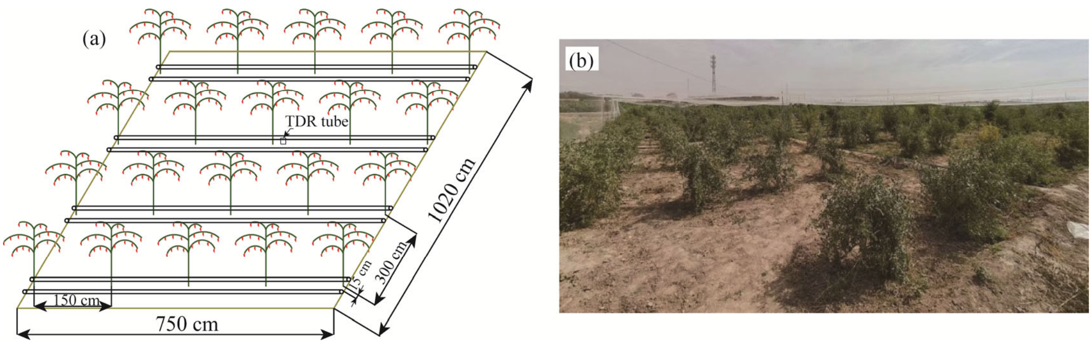

Fig. 2 Experimental plot design (a) and field map (b) of study area. TDR, time domain reflectometer.

2.3 Data analysis

2.3.1 Soil moisture content

A portable time domain reflectometer (TDR, PICO-BT, IMKO, Ettlingen, Germany) was used to measure soil moisture content. Measurements were taken once every 7 d, both before and after irrigation and rainfall events. In addition, soil moisture content was checked regularly using the drying method.

Water consumption (WC; mm) during the growth period of wolfberry was calculated as follows:

$$\mathrm{WC} = P + W_2 - W_1 + I + K - R - D_\mathrm{P}\,, \tag{1}$$

where P is the rainfall during the growth period (mm); W_2 is the annual water storage (mm) of the 0–120 cm soil layer after harvest; W_1 is the annual water storage in the 0–120 cm soil layer (mm) at the beginning of the experiment; I is the irrigation amount (mm); K is the groundwater recharge (mm); R is the runoff (mm); and D_p is the deep leakage (mm). Because the groundwater depth in the test area was below 5 m, the terrain was flat, and the single rainfall amount was small; therefore, K, R, and D_p can be ignored.

2.3.2 Growth index

Three plants of wolfberry with the same growth trend were randomly selected from each plot, and plant height, stem diameter, and crown width were measured at vegetative growth, full flowering, summer peak fruit, and autumn peak fruit stages. Steel tape was used to measure the plant height (cm) from the ground to the top of wolfberry.

Vernier caliper was used to measure the stem diameter (mm) of wolfberry approximately. The

crown width (cm) of wolfberry naturally distributed in the east-west direction (E-W) and south-north direction (N-S) was measured using steel tape.

2.3.3 Yield and constituent elements

After ripening from the end of July to August, we picked wolfberry every 7 d, dried naturally, weighed, and converted to yield (Y; kg/hm^2) per unit area according to the plot area (only the production in 2022 was counted).

Three wolfberry plants were grown in each plot. After each fresh fruit was harvested, we dried and weighed the plants. Dry weight (g) of multiple harvests was summed and the average value was calculated, which represents dry weight per plant.

Three wolfberry plants were grown in each plot. One hundred berries were randomly selected each time and their weight was immediately measured, which represented fresh weight (FW; g). The dry weight (DW; g) of samples was determined after drying. The dry weight percentage was calculated as follows.

$$\text{Dry/fresh percentage}=\text{DW}/\text{FW}\times 100\%. \tag{2}$$

2.3.4 Irrigation water use efficiency (IWUE), WUE, partial factor productivity of nitrogen (PFPN), and NUE

IWUE (kg/(hm^2·mm)) was calculated as follows:

$$\text{IWUE} = Y / I , \tag{3}$$

where Y is the yield (kg/hm^2).

WUE (kg/(hm^2·mm)) was calculated as follows:

$$\text{WUE} = Y / \text{WC} . \tag{4}$$

PFPN (kg/kg) was calculated as follows:

$$\text{PFPN} = Y / N , \tag{5}$$

where N is the nitrogen application rate (kg/hm^2).

NUE (kg/kg) was calculated as follows:

$$\text{NUE} = \left(Y_{\text{NPK}} - Y_{\text{PK}}\right) / N , \tag{6}$$

where Y_{NPK} is the dried fruit yield of wolfberry (kg/hm^2) in the nitrogen application plot; and Y_{PK} is the yield (kg/hm^2) of dried wolfberry fruit without nitrogen application.

2.4 Principal component analysis (PCA)

PCA is a widely used dimensionality reduction method, which can convert multiple indicators into several principal components (Li et al., 2021a).

2.5 Statistical analysis

Microsoft Excel v.2010 was used for data processing, Origin v.2021 software was used for mapping, and SPSS Statistics v.27.0 software was used for variance analysis, significance test (significance at P<0.05 level), and PCA.

3 Results

3.1 Effects of water and nitrogen application levels on the growth of wolfberry

3.1.1 Plant height

Water and nitrogen application levels had significant effects on plant height at different wolfberry growth stages (P<0.01). The interaction of water and nitrogen also had significant effects on plant height at the summer and autumn peak fruit stages in both years (P<0.01), as well as on plant height at the full flowering stage in 2022 (P<0.05; Fig. 3). Under different water and nitrogen application levels, growth rate of wolfberry varied at different growth stages. From vegetative growth stage to full flowering stage, growth rate was the fastest, with an average of 24.69% over two years. However, from summer to autumn peak fruit stages, growth rate was the slowest, with an average of 8.50%. Under the same water level, plant height of wolfberry at each growth stage first increased and then decreased with increasing nitrogen application. The order of nitrogen

effects from the highest to the lowest on plant height was N2>N3>N1>N0. Specifically, compared with N0, plant height increased by 7.85% for N1, 15.33% for N2, and 11.83% for N3. At the same nitrogen application level, plant height of wolfberry first increased and then decreased at each growth stage with an increase in water deficit. The order of water deficit effects from the highest to the lowest on plant height was W1>W0>W2>W3. On average, W1 showed an increase of 3.03% compared with W0, whereas W2 and W3 decreased by 6.84% and 19.87%, respectively, compared with W0. Among all treatments, plant height of W1N2 treatment was the highest, measuring 19.67% higher than that of W0N0 treatment. In conclusion, proper irrigation and nitrogen application can promote the growth of wolfberry. Vegetative growth and full flowering stages were more sensitive to water and nitrogen applications than the other growth stages.

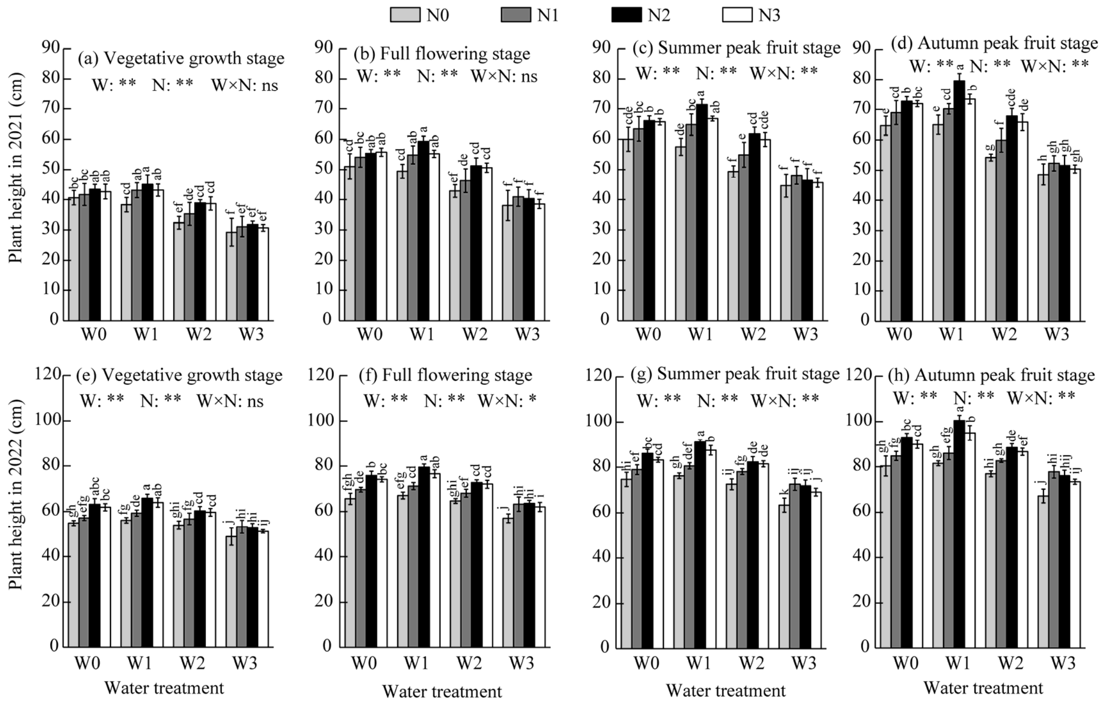

Fig. 3 Effects of water (W) and nitrogen (N) application levels on plant height at different wolfberry growth stages in 2021 (a–d) and 2022 (e–h). Different lowercase letters indicate significant differences in plant height under different water and nitrogen treatments at $P<0.05$ level. W×N, water and nitrogen interaction. **, $P<0.01$ level; *, $P<0.05$ level; ns, no significant difference; W0, 75%–85% field water capacity (θ_f); W1, 65%–75% θ_f; W2, 55%–65% θ_f; W3, 45%–55% θ_f; N0, 0 kg/hm^2; N1, 150 kg/hm^2; N2, 300 kg/hm^2; N3, 450 kg/hm^2. Bars are standard errors. The abbreviations and treatments are the same as in the following figures and tables.

3.1.2 Stem diameter

Water and nitrogen application levels had significant effects on stem diameter at different growth stages ($P<0.01$). In addition, their interaction had significant effects on stem diameter at the summer and autumn peak fruit stages in 2021 as well as at various growth stages in 2022 ($P<0.05$; Fig. 4). Under different water and nitrogen levels, stem diameter increased by an average of 33.30% from vegetative growth to full flowering stages over 2 a, and from summer to autumn peak fruit stage, stem diameter increased by an average of 9.65%. Stem diameter first increased and then decreased with increasing nitrogen application, reaching a maximum under N2 treatment. It decreased with a decrease in irrigation amount and reached a maximum under W0 treatment. Among all treatments, stem diameter of W0N2 treatment was the largest, which was 22.34% higher than that of W0N0 treatment. In conclusion, appropriate nitrogen application and irrigation can promote stem diameter growth at different growth stages of wolfberry, particularly at vegetative growth and full flowering stages.

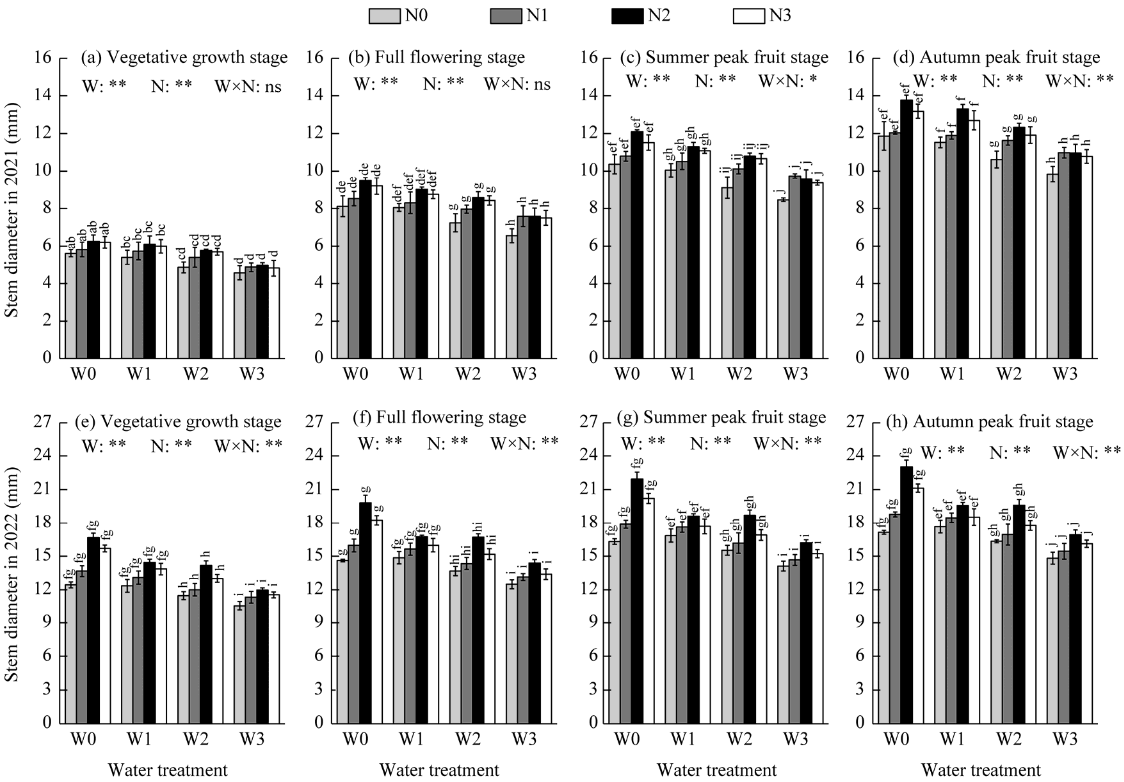

Fig. 4 Effects of water (W) and nitrogen (N) application levels on stem diameter at different wolfberry growth stages in 2021 (a–d) and 2022 (e–h). Different lowercase letters indicate significant differences in stem diameter under different water and nitrogen treatments at $P<0.05$ level. **, $P<0.01$ level; *, $P<0.05$ level; ns, no significant difference; Bars are standard errors.

3.1.3 Crown width

Water and nitrogen application levels had significant effects on crown width at each growth stage ($P<0.01$), and their interaction had significant effects on crown width at the summer peak fruit stage and autumn peak fruit stage in 2021, as well as at each growth stage (except for vegetative growth stage) in 2022 ($P<0.05$; Table 1). Under different water and nitrogen application levels, crown width increased at the fastest rate from vegetative to full flowering stage but growth rate was the slowest from the summer to autumn peak fruit stage, and east-west crown width was significantly larger than that of north-south crown width. Under W0 and W1 treatments, crown widths of north-south and east-west increased with increasing nitrogen application at each growth stage. Under treatments of W2 and W3, crown widths of north-south and east-west first increased and then decreased with increasing nitrogen application, showing the order of N2>N3>N1>N0 at each growth stage. With the same amount of nitrogen applied, crown widths of N-S and E-W at each growth stage first increased and then decreased with the intensification of water deficit, as the order of W1>W0>W2>W3. The maximum crown widths of north-south and east-west appeared in W1N3 treatment, which increased by 26.03% and 21.88%, respectively, compared with W0N0 treatment. The lowest values were found in W3N0 treatment, which decreased by 20.15% and 19.92%, respectively, compared with W0N0 treatment. Water-nitrogen coupling effect of W1N3 treatment was the best in terms of crown width.

3.2 Effects of water and nitrogen application levels on yield and yield components of wolfberry

Water and nitrogen application levels significantly affected the yield and yield components of wolfberry ($P<0.01$), and their interaction significantly affected the dry weight per hundred grains,

Table 1 Effects of water (W) and nitrogen (N) application levels on crown width at different wolfberry growth stages in 2021 and 2022

Year	Treatment	Vegetative growth stage		Full flowering stage		Summer peak fruit stage		Autumn peak fruit stage	
		N-SCW (cm)	E-WCW (cm)	N-SCW (cm)	E-WCW (cm)	N-SCW (cm)	E-WCW (cm)	N-SCW (cm)	E-WCW (cm)
2021	W0N0	37.21±1.13^{cde}	38.47±1.75^{d}	49.35±1.98^{cde}	52.68±1.46^{d}	58.45±2.39^{fg}	60.79±1.53^{ef}	61.96±3.28^{fg}	64.29±1.48^{f}
	W0N1	38.58±1.70^{bc}	40.12±1.25^{bcd}	51.70±1.1.57^{bcd}	54.67±2.07^{bcd}	60.42±1.70^{def}	63.08±2.24^{de}	64.13±1.31^{def}	66.47±1.74^{ef}
	W0N2	40.19±1.24^{abc}	41.52±1.23^{abc}	54.83±2.68^{ab}	55.90±1.74^{abcd}	64.22±2.66^{bcd}	65.88±2.43^{bcd}	68.36±2.93^{bcd}	72.02±1.64^{bcd}
	W0N3	40.40±2.05^{abc}	41.87±1.42^{ab}	56.04±2.63^{ab}	57.31±1.92^{abc}	66.75±1.70^{bc}	68.75±3.13^{abc}	71.06±2.36^{bc}	73.72±0.73^{bc}
	W1N0	37.34±2.34^{cde}	38.58±1.14^{b}	51.59±3.01^{bcd}	53.26±2.88^{cd}	59.76±1.13^{efg}	61.86±2.93^{de}	63.29±1.06^{efg}	66.49±4.81^{ef}
	W1N1	38.15±1.95^{bcd}	39.48±1.11^{bcd}	52.49±2.22^{bc}	54.89±3.24^{bcd}	61.13±2.00^{def}	64.46±2.78^{de}	65.67±1.63^{def}	68.47±3.68^{de}
	W1N2	40.94±1.25^{ab}	42.14±1.81^{ab}	56.34±3.79^{ab}	58.52±2.07^{ab}	67.64±3.02^{ab}	69.64±0.87^{ab}	72.02±2.53^{b}	75.41±0.83^{ab}
	W1N3	42.03±1.37^{a}	43.30±1.59^{a}	58.77±3.46^{a}	59.97±2.50^{a}	70.89±2.44^{a}	72.22±1.76^{a}	76.96±2.34^{a}	78.96±1.75^{a}
	W2N0	34.31±1.29^{ef}	34.65±1.08^{e}	46.94±3.52^{defg}	46.77±2.83^{ef}	52.89±2.97^{hi}	53.59±3.78^{gh}	55.69±3.16^{hi}	58.36±1.60^{gh}
	W2N1	35.36±1.41^{def}	35.22±1.34^{e}	48.40±2.53^{cdef}	48.04±1.66^{e}	56.45±1.67^{gh}	57.22±1.27^{fgh}	59.28±1.94^{gh}	60.62±0.76^{g}
	W2N2	39.00±1.50^{abc}	39.00±1.50^{cd}	53.59±2.02^{abc}	53.09±2.66^{cd}	63.05±1.39^{cde}	65.93±2.22^{bcd}	67.36±2.56^{cde}	71.03±3.04^{cd}
	W2N3	38.79±1.82^{bc}	41.13±2.39^{abcd}	52.31±2.1.98^{bcd}	54.81±1.32^{bcd}	60.82±2.02^{def}	64.82±1.80^{cde}	65.21±1.75^{def}	69.15±2.81^{de}
	W3N0	30.01±3.06^{g}	32.95±1.33^{e}	38.97±2.70^{h}	42.27±2.35^{g}	43.49±2.62^{k}	46.82±1.52^{j}	44.97±2.57^{j}	48.07±1.53^{i}
	W3N1	33.00±1.24^{fg}	34.33±1.43^{e}	44.92±2.75^{efg}	45.42±2.29^{efg}	49.98±1.42^{ij}	52.64±2.46^{hi}	52.98±1.43^{ij}	54.98±1.42^{h}
	W3N2	33.09±1.17^{fg}	33.76±1.32^{e}	43.66±3.65^{efg}	44.66±2.65^{efg}	48.80±3.19^{ij}	50.13±1.13^{hij}	52.47±3.22^{ij}	55.63±1.15^{h}
	W3N3	32.60±1.75^{fg}	34.03±1.48^{e}	42.68±4.00^{gh}	43.21±2.11^{fg}	47.69±3.67^{j}	48.99±1.54^{ij}	49.81±2.72^{j}	50.84±1.41^{i}
2022	W0N0	54.63±0.94^{ef}	57.63±1.98^{de}	65.13±0.81^{gh}	68.17±2.69^{ef}	71.11±1.14^{gh}	75.18±1.86^{fg}	74.16±1.77^{fg}	78.83±2.19^{ef}
	W0N1	58.13±1.94^{de}	59.80±1.88^{cd}	69.13±1.65^{efg}	71.53±2.06^{de}	75.51±1.94^{efg}	77.91±1.95^{ef}	78.89±1.69^{ef}	81.71±2.32^{de}
	W0N2	61.06±3.06^{cd}	62.73±1.99^{def}	74.09±2.97^{cd}	75.71±1.60^{bcd}	81.49±322^{cd}	83.56±3.14^{cd}	85.51±3.93^{c}	87.76±2.85^{c}
	W0N3	63.75±1.18^{bc}	63.75±2.10^{bc}	76.86±1.23^{bc}	77.33±2.53^{bc}	85.36±1.50^{bc}	87.19±2.77^{bc}	90.49±1.56^{b}	92.31±2.03^{b}
	W1N0	56.63±1.67^{e}	60.15±1.55^{bcd}	67.63±1.64^{fg}	69.23±1.65^{ef}	74.26±1.08^{fg}	76.95±1.58^{ef}	78.22±1.26^{ef}	81.48±2.46^{de}
	W1N1	60.65±3.03^{cd}	61.79±1.39^{bcd}	72.69±2.95^{de}	71.53±2.55^{de}	79.99±3.90^{de}	79.51±1.60^{def}	83.99±3.71^{cd}	83.18±2.09^{cde}
	W1N2	65.23±1.79^{ab}	64.57±2.59^{ab}	79.29±3.01^{b}	79.95±1.93^{ab}	88.22±2.60^{b}	90.31±2.78^{b}	93.98±3.28^{b}	95.14±3.27^{b}
	W1N3	68.33±2.25^{a}	68.42±2.21^{a}	83.85±2.58^{a}	84.29±1.87^{a}	93.86±2.98^{a}	95.50±3.07^{a}	100.16±3.73^{a}	101.89±3.18^{a}
	W2N0	50.63±1.43^{gh}	53.36±2.28^{fg}	60.07±1.72^{ij}	62.81±3.20^{gh}	65.95±3.65^{ij}	67.51±2.42^{h}	68.87±3.79^{hi}	70.60±2.69^{g}
	W2N1	52.03±0.68^{fg}	55.49±4.15^{ef}	61.95±1.70^{hi}	66.15±4.79^{fg}	68.23±1.76^{hi}	72.06±2.28^{g}	71.33±1.48^{gh}	75.63±1.50^{f}
	W2N2	57.89±2.93^{de}	60.35±3.32^{bcd}	69.52±3.01^{ef}	73.20±3.64^{cde}	76.69±2.96^{ef}	80.93±1.89^{de}	80.39±2.68^{de}	84.87±2.56^{cd}
	W2N3	54.88±2.36^{ef}	58.15±3.23^{de}	65.44±2.00^{fgh}	69.29±3.17^{ef}	71.44±2.29^{gh}	76.66±3.83^{ef}	74.70±2.55^{fg}	79.81±4.22^{ef}
	W3N0	46.98±1.95^{h}	48.89±2.56^{h}	55.44±1.77^{k}	56.40±2.74^{i}	57.42±2.26^{l}	59.84±3.18^{j}	59.57±2.53^{k}	62.01±3.45^{i}
	W3N1	52.16±2.84^{fg}	52.98±1.93^{fgh}	61.57±3.59^{hi}	62.98±1.74^{gh}	65.26±3.58^{ij}	66.59±1.94^{hi}	68.84±2.45^{hi}	69.46±1.91^{gh}
	W3N2	50.00±2.20^{gh}	51.47±1.58^{fgh}	59.18±2.13^{hi}	60.27±3.22^{hi}	62.78±2.41^{jk}	65.66±3.12^{hi}	66.48±1.71^{ij}	68.42±3.09^{gh}
	W3N3	48.23±2.40^{gh}	49.78±1.46^{gh}	57.11±2.53^{jk}	57.36±1.88^{i}	59.25±2.85^{kl}	62.41±1.86^{ij}	62.60±2.51^{jk}	64.82±1.52^{hi}

Analysis of variance									
2021	W	**	**	**	**	**	**	**	**
	N	**	**	**	**	**	**	**	**
	W×N	ns	ns	ns	ns	*	**	**	**
2022	W	**	**	**	**	**	**	**	**
	N	**	**	**	**	**	**	**	**
	W×N	**	ns	**	**	**	**	**	**

Note: Different lowercase letters within the same year indicate significant differences in crown width under different water and nitrogen treatments at $P<0.05$ level. **, $P<0.01$ level; *, $P<0.05$ level; ns, no significant difference; N-S, north-south; E-W, east-west. Mean±SD; $n=3$.

dry:fresh ratio, and yield of wolfberry (P<0.05; Table 2). Under the same water level, dry weight per plant and yield of wolfberry first increased and then decreased with increasing nitrogen application, demonstrating that the highest yield was observed with N2, followed by N3, N1, and N0. Under W0 treatment, dry weight per hundred grains and dry weight percentage increased with an increase in nitrogen application, and dry weight per hundred grains and dry weight percentage first increased and then decreased with an increase in nitrogen application under other water levels. Under the same nitrogen application level, yield and yield components of wolfberry first increased and then decreased with increasing water deficit, showing the order of W1>W0>W2>W3. All of the dry weight per plant, dry weight per hundred grains, dry weight percentage, and yield of wolfberry were the highest in W1N2 treatment, increasing by 47.55%, 47.85%, 16.31%, and 47.55%, respectively, compared with W0N0 treatment. Dry weight per plant, dry weight per hundred grains, and yield with W3N0 treatment were the lowest, which decreased by 23.04%, 22.74%, and 23.04%, respectively, compared with those of W0N0 treatment. W3N3 treatment had the smallest dry weight percentage, which was 6.42% lower than that of W0N0 treatment. In conclusion, under mild water deficit, excessive nitrogen application can inhibit wolfberry yield.

Table 2 Effects of water (W) and nitrogen (N) application levels on yield and yield components of wolfberry

Treatment	Dry weight per plant (g)	Dry weight per hundred grains (g)	Dry weight percentage (%)	Yield (kg/hm^2)
W0N0	680.00±34.64^{f}	13.94±0.41^{gh}	23.67±0.55^{cde}	1777.78±90.56^{f}
W0N1	783.33±47.26^{de}	15.18±0.42^{de}	23.97±0.96^{def}	2047.93±177.96^{de}
W0N2	943.33±60.00^{ab}	17.10±0.12^{c}	24.71±0.98^{cd}	2466.23±123.55^{ab}
W0N3	850.00±68.07^{cd}	17.59±0.28^{c}	24.95±0.31^{c}	2222.22±156.86^{cd}
W1N0	710.00±30.00^{ef}	14.48±0.4e^{fg}	23.93±0.35^{def}	1856.21±117.89^{ef}
W1N1	820.00±26.46^{cd}	15.82±0.55^{d}	24.14±0.94^{cde}	2143.79±69.17^{cd}
W1N2	1003.33±50.33^{a}	20.61±0.21^{a}	27.53±1.04^{a}	2623.09±131.59^{a}
W1N3	900.00±50.00^{bc}	19.42±0.54^{b}	26.29±0.42^{b}	2352.94±130.72^{bc}
W2N0	650.00±51.96^{fg}	12.55±0.33^{i}	22.86±0.50^{fg}	1699.35±135.85^{fg}
W2N1	696.67±15.28^{f}	13.32±0.34^{h}	23.14±0.44^{efg}	1821.35±39.94^{f}
W2N2	843.33±32.15^{cd}	15.09±0.93^{def}	23.57±0.58^{def}	2204.79±84.04^{cd}
W2N3	800.00±65.57^{d}	14.34±0.36^{fg}	23.32±0.49^{efg}	2091.50±171.44^{d}
W3N0	523.33±40.41^{h}	10.77±0.34^{j}	22.77±1.00^{fg}	1368.19±105.66^{h}
W3N1	540.00±52.92^{h}	11.23±0.23^{j}	22.19±0.60^{g}	1411.76±138.34^{h}
W3N2	630.00±10.00^{fg}	12.39±0.75^{i}	22.24±0.29^{g}	1647.06±26.14^{fg}
W3N3	576.67±45.09^{gh}	12.10±0.44^{i}	22.15±0.99^{g}	1507.63±117.89^{gh}
Analysis of variance				
W	**	**	**	**
N	**	**	**	**
W×N	ns	**	**	*

Note: Different lowercase letters indicate significant differences in yield and yield components under different water and nitrogen treatments at P<0.05 level. **, P<0.01 level; *, P<0.05 level; ns, no significant difference. Mean±SD; n=3.

3.3 Effects of water and nitrogen application levels on WUE and NUE of wolfberry

Water and nitrogen application levels had significant effects on WUE and NUE (P<0.01), and their interaction had significant effects on WC, WUE, and PFPN (P<0.01; Table 3). Under the same water level, IWUE, WUE, and NUE first increased and then decreased with an increase in nitrogen application, whereas PFPN decreased with an increase in nitrogen application. Under the same nitrogen application, under N2 and N3 treatments, IWUE and WUE first increased and then decreased with an increase in water deficit, whereas under other treatments, PFPN and NUE first

Table 3 Effects of water (W) and nitrogen (N) application levels on irrigation water use efficiency (IWUE), water (WUE), partial factor productivity of nitrogen (PFPN), and nitrogen use efficiency (NUE) of wolfberry

Treatment	*I* (mm)	WC (mm)	IWUE (kg/(hm^2•mm))	WUE (kg/(hm^2•mm))	PFPN (kg/kg)	NUE (kg/kg)
W0N0	452.84±10.00^a	517.17±8.97^a	3.93±0.20^i	3.44±0.18^g	-	-
W0N1	414.34±22.55^b	466.43±20.54^{bc}	4.94±0.43^{gh}	4.39±0.38^{ef}	13.65±1.19^a	1.80±0.31^{ab}
W0N2	372.16±20.31^c	481.00±10.16^b	6.63±0.33^{de}	5.13±0.26^{cd}	8.22±0.41^{de}	2.29±0.51^a
W0N3	392.60±30.25^{bc}	444.48±30.00^c	5.66±0.40^{fg}	5.00±0.35^{cd}	4.94±0.35^f	0.99±0.20^{bc}
W1N0	384.91±4.83^c	453.82±6.51^c	4.82±0.20^h	4.09±0.17^f	-	-
W1N1	391.40±16.32^{bc}	454.30±13.46^c	5.48±0.18^{gh}	4.72±0.15^{de}	14.29±0.46^a	1.92±0.27^{ab}
W1N2	315.41±10.14^{de}	383.90±8.43^d	8.32±0.42^a	6.83±0.34^a	8.74±0.44^{cd}	2.56±0.49^a
W1N3	333.27±4.00^d	394.08±4.32^d	7.06±0.39^{cd}	5.97±0.33^b	5.23±0.29^f	1.10±0.41^{bc}
W2N0	316.98±2.65^{de}	372.03±2.00^d	5.36±0.43^{gh}	4.57±0.37^{def}	-	-
W2N1	290.04±10.48^{ef}	379.58±11.37^d	6.28±0.14^{ef}	4.80±0.11^{de}	12.14±0.27^b	0.81±0.19^{bc}
W2N2	260.52±10.36^g	331.88±3.51^e	8.46±0.32^a	6.64±0.25^a	7.35±0.28^e	1.68±0.21^{ab}
W2N3	274.46±5.00^{fg}	385.15±4.64^d	7.62±0.62^{bc}	5.43±0.45^{bc}	4.65±0.38^f	0.87±0.23^{bc}
W3N0	249.06±5.51^{gh}	294.33f±6.00^g	5.49±0.42^{gh}	4.65±0.36^{def}	-	-
W3N1	227.89±7.89^{hi}	279.43±9.48^g	6.20±0.61^{ef}	5.05±0.50^{cd}	9.41±0.92^c	0.29±0.03^c
W3N2	204.69±9.86^i	278.57±6.76^g	8.05±0.13^{ab}	5.92±0.12^b	5.49±0.09^f	0.93±0.44^{bc}
W3N3	215.64±5.43^i	309.03±10.21^f	6.99±0.55^{cd}	4.88±0.07^{cde}	3.35±0.26^g	0.31±0.29^c
Analysis of variance						
W	**	**	**	**	**	**
N	**	**	**	**	**	**
W×N	ns	**	ns	**	**	ns

Note: *I*, irrigation; WC, water consumption. Different lowercase letters indicate significant differences in WUE and NUE under different water and nitrogen treatments at $P<0.05$ level. **, $P<0.01$ level; ns, no significant difference. Mean±SD; $n=3$. -, no value;

increased and then decreased with an increase in water deficit. Among all treatments, W2N2 had the largest IWUE (8.46 kg/(hm^2•mm)), W1N2 had the largest WUE (6.83 kg/(hm^2•mm)), and NUE (2.56 kg/kg), and W1N1 had the largest PFPN (14.29 kg/kg).

3.4 Comprehensive analysis of wolfberry under different water and nitrogen application levels

3.4.1 Correlation analysis

A correlation analysis was conducted for plant height, stem diameter, north-south crown width, east-west crown width, dry weight per plant, dry weight per hundred grains, dry weight percentage, irrigation amount, nitrogen application rate, WC, IWUE, WUE, PFPN, NUE, and yield under different water and nitrogen application levels (Fig. 5). The correlation coefficients among indicators were greater than 0.600, indicating a highly significant correlation among selected indicators.

3.4.2 PCA

PCA revealed that variance contribution percentages of PC1 (principal component) and PC2 were 61.58% and 23.25%, respectively, and the cumulative variance was 84.82% (Fig. 6). PC1 contained 61.58% of the total variation, reflecting the effects of 12 indices including plant height, stem diameter, north-south crown width, east-west crown width, dry weight per plant, dry weight per hundred grains, dry weight percentage, nitrogen application rate, yield, PFPN, NUE, and WUE. PC2 explained 23.25% of the total variation, reflecting the effects of 3 indices including irrigation, WC, and IWUE. According to the ranking of comprehensive scores for each treatment (Fig. 7), W1 had the highest comprehensive score among the four levels of water application. Similarly, among the four nitrogen levels, N2 had the highest score. Among all the treatments, W1N2 treatment had the highest score.

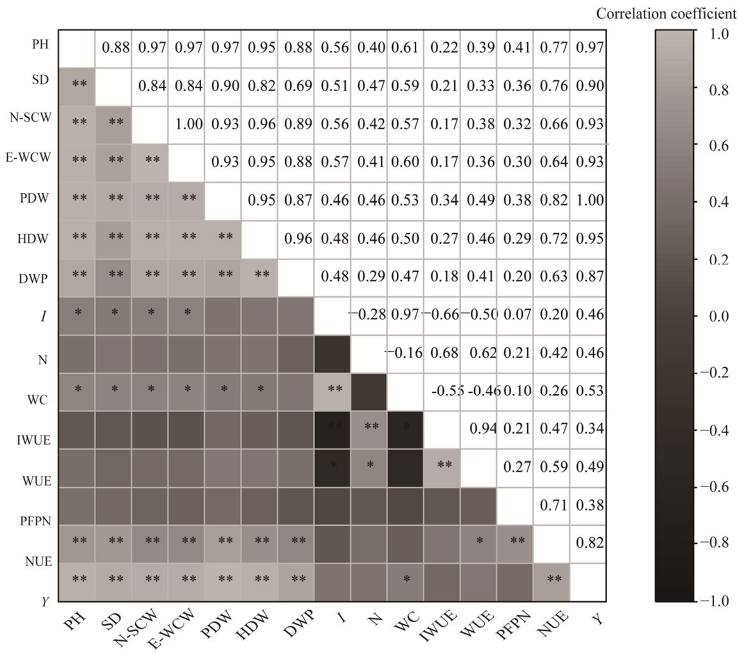

Fig. 5 Correlation analysis between parameters under different water and nitrogen application levels. PH, plant height; SD, stem diameter; N-SCW, north-south crown width; E-WCW, east-west crown width; PDW, dry weight per plant; HDW, dry weight per hundred grains; DWP, dry weight percentage; *I*, irrigation; N, nitrogen; WC, water consumption; IWUE, irrigation water use efficiency; WUE, water use efficiency; PFPN, partial factor productivity of nitrogen; NUE, nitrogen use efficiency; *Y*, yield. **, *P*<0.01 level; *, *P*<0.05 level. The abbreviations are the same as in the following figures.

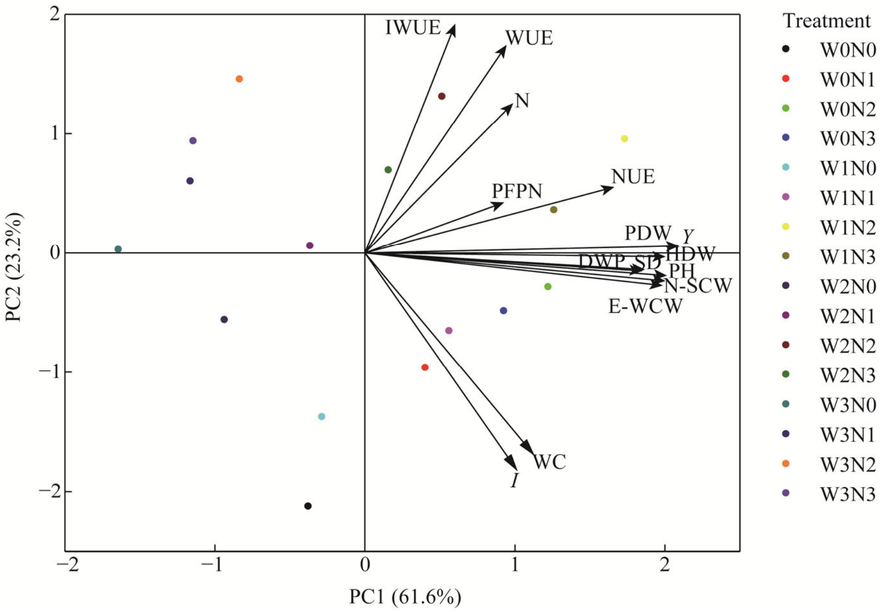

Fig. 6 Principal component analysis (PCA) among different indices of wolfberry. PC, principal component.

4 Discussion

4.1 Effects of water and nitrogen application levels on growth of wolfberry

Plant height, stem diameter, and crown width are important indicators of wolfberry growth (Wozniak et al., 2012). An appropriate water:nitrogen ratio can provide suitable environmental

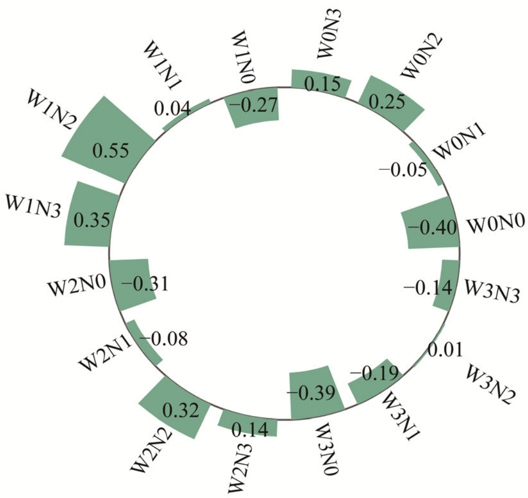

Fig. 7 Comprehensive scores of wolfberry using multiple indices under different water (W) and nitrogen (N) application levels

conditions for wolfberry and promote its healthy growth (Li et al., 2021b). In this study, it was found that east-west crown width of wolfberry was generally larger than that of north-south crown width, which may be because the photosynthetically active radiation absorbed by east-west crown width was greater than that of north-south crown width, and the increased photosynthetic intensity was more conducive to plant elongation (Wozniak et al., 2012). This study also found that plant height, stem diameter, and crown width of wolfberry increased at the fastest rate from the vegetative to full flowering stages, while the growth rate was the slowest from the summer to autumn peak fruit stages. The above phenomenon further indicates that wolfberry is more sensitive to water and nitrogen at the vegetative growth and full flowering stages than the other growth stages. At the same time, in this study, plant height of wolfberry first increased and then decreased with the decrease of irrigation amount (204.69–452.84 mm). However, Yin et al. (2018) showed that with the decrease in irrigation amount (210.00–510.00 mm), the plant height of wolfberry first increased, then decreased, and then increased. The reason for the differences between the two researches may be related to the soil texture of study area (sandy loam and lime soil) and the depth of groundwater (5 and 1 m, respectively). Compared with sandy loam, lime soil is less permeable to water and gas. Root rot occurs in the presence of excessive irrigation. When there is insufficient irrigation and the groundwater depth is shallow, wolfberry can be supplied with groundwater for growth. A certain degree of water deficit and reduced nitrogen application is conducive to the growth of crop, and too little or too much water and nitrogen input causes crop growth retardation to a certain extent (De et al., 2012; Song et al., 2019). This study showed that plant height of wolfberry at each growth stage first increased and then decreased with a decrease in irrigation and nitrogen application. Stem diameter decreased with a decrease in irrigation amount, first increased, and then decreased with a decrease in nitrogen application. Water is the driving force behind the expansion and growth of plant cells. Suitable soil water status can promote the migration, absorption, and utilization of nitrogen and other nutrients and promote the growth of crop roots and canopy (Zhang et al., 2015b). Dissolved nitrogen can reduce soil water evaporation, enhance leaf photosynthesis, and promote the growth of plant stems and leaves (Gheith et al., 2022; Yang et al., 2023). This study also concluded that the interaction of water and nitrogen significantly affected plant height, stem diameter, and crown width of wolfberry at the summer and autumn peak fruit stages.

4.2 Effects of water and nitrogen application levels on yield and yield components of wolfberry

Appropriate input of water and nitrogen during crop growth period is conducive to promoting the

absorption of nutrients and water by crops and increasing yield (Tang et al., 2010; Wang et al., 2015). Appropriate nitrogen application increases the chlorophyll content of leaves, promotes the accumulation of photosynthetic products, and facilitates the formation of yield (Tang et al., 2010). Dry weight per plant, dry weight per hundred grains, and the dry weight percentage were the main yield components of wolfberry. This study showed that under the same water treatment, dry weight per plant and yield first increased and then decreased with a decrease in nitrogen application. Under W0 treatment, dry weight per hundred grains and dry weight percentage increased with increasing nitrogen application. Under W1, W2, and W3 treatments, dry weight per hundred grains, and dry weight percentage first increased and then decreased with increasing nitrogen application. Under the same nitrogen application, yield of wolfberry first increased and then decreased with the increase in irrigation amount (204.69–452.84 mm), while, Wang et al. (2015) found that when the upper limit of irrigation was 95% θ_f, the yield of wolfberry increased as the lower limit of irrigation increased from 30% to 70% θ_f. The reasons for the differences between the two studies may be because of the smaller upper and lower irrigation gradients (10% θ_f) used in this study, which resulted in longer irrigation durations. Frequent irrigation aggravates ion flow, particularly the flow of available nutrients, which leads to a decline in soil fertility in the root layer. This decrease in fertility hinders the ability of crops to obtain sufficient water and fertilizer, ultimately limiting the accumulation of dry matter (Gheith et al., 2022). Under a suitable water and nutrient environment, it is beneficial to enhance the assimilation and transport capacity of soil nitrogen, improve the vitality of plant roots, and then increase the number of enzymes synthesized in the photosynthetic system, accelerate the net photosynthetic rate, and improve dry matter accumulation capacity (Li et al., 2019a; Fu et al., 2021). This study also showed that the yield of wolfberry first increased and then decreased with decreasing in irrigation and nitrogen levels. All of the dry weight per plant, dry weight per hundred grains, dry weight percentage, and yield of wolfberry were the highest in W1N2 treatment, increasing by 47.55%, 47.85%, 16.31%, and 47.55%, respectively, compared with W0N0 treatment. However, Liu et al. (2021) found that yield of wolfberry first increased and then decreased with a decrease in irrigation and gradually decreased with a decrease in nitrogen application (207–345 kg/hm^2). The reasons for the differences between the two studies were mainly attributed to the following two aspects: on the one hand, there were differences in the nitrogen application rates, and the lower nitrogen application rate may not have reached the nitrogen requirement threshold of wolfberry; on the other hand, it was related to the basic nutrient status of the experimental site (the total nitrogen contents were 1.62 and 0.36 g/kg, respectively). When the soil's inherent nitrogen content was low, the growth of wolfberry was more dependent on exogenous nitrogen, thus increasing the nitrogen application amount.

4.3 Effects of water and nitrogen application levels on WUE and NUE of wolfberry

WUE and NUE can directly reflect the effects of crop water and nitrogen inputs (Rueda-Ayala et al., 2019; Liao et al., 2022). Insufficient nitrogen application in irrigation water weakens the absorption and utilization of nitrogen by plants, and excessive nitrogen application in irrigation water causes root rot in plants, which is not conducive to improving WUE and NUE (Yan et al., 2017; Wu et al., 2020). IWUE is the ratio of yield to irrigation amount and is often used to measure the degree of utilization of irrigation water (Zhao et al., 2021). WUE is the ratio of yield to water consumption, which reflects the energy conversion efficiency of plant production and evaluates the suitability of plant growth (Li et al., 2019a). This study found that IWUE and WUE of wolfberry were higher under mild to moderate water deficit than those under full irrigation and severe water deficit, which was mainly because excessive irrigation causes a large amount of water to accumulate in the soil, resulting in nutrient loss. Insufficient irrigation leads to stomatal closure of crop leaves, damages to the chloroplast structure, weakens photosynthesis, and reduces dry matter accumulation (Li et al., 2020b; Zhang et al., 2020). PFPN is the ratio between yield and nitrogen application, which reflects the combined effect of local soil basic nutrient level and fertilizer application amount (Lin et al., 2019). NUE is the ratio between the yield difference in

the nitrogen and the no-nitrogen application zones, which can reflect the utilization rate of nitrogen fertilizer (Kumar et al., 2022). The results showed that PFPN of wolfberry decreased with increasing nitrogen application. Similar conclusions were also found by Abdalhi et al. (2016) in the study of corn and cucumber in Jiangsu, China, and Hao et al. (2022) in the study of apple in the Loess Plateau, China. Visibly, there is a threshold for crop nitrogen absorption, when the nitrogen application rate is too high. PFPN will be significantly reduced, resulting in a serious waste of nitrogen resources (Ogle et al., 2016). In this study, it was concluded that PFPN of wolfberry first increased and then decreased with a decrease in irrigation amount, while, Li et al. (2022b) found that under the same nitrogen application amount, PFPN of tomato gradually decreased with a decrease in irrigation amount. The reason for the difference may be due to differences in crop types. In general, the water requirement during the growth period of wolfberry was 60%–70% θ_f, and the maximum irrigation water amount (75%–85% θ_f) in this experiment exceeded the appropriate water requirement for wolfberry. The water requirement of tomato is 60%–80% θ_f, and the maximum irrigation amount (60%–65% θ_f) set by Li et al (2022b) has not reached the appropriate water requirement for tomato. Appropriate water and nitrogen stress can improve drought resistance, water and fertilizer absorption capacity of plants, yield, and NUE (Badr et al., 2012). This study also found that NUE was the highest under mild water deficit (65%–75% θ_f) and moderate nitrogen application (300 kg/hm^2).

The water and nitrogen application levels of wolfberry during the whole growth stage was studied in this study. However, the water and nitrogen requirements of wolfberry at different growth stages are different, so water and nitrogen application should be carried out at different growth stages of wolfberry in the future. In addition, wolfberry is a perennial economic forest, and only the research in water and nitrogen was conducted for the 2- and 3-a wolfberry. Subsequent studies should be carried out on water and nitrogen regulation threshold for different planting years of wolfberry.

5 Conclusions

Irrigation and nitrogen application significantly affected the growth of wolfberry. The yield, dry weight per plant, dry weight per hundred grains, and dry weight percentage of wolfberry were the highest under W1N2 treatment, which were 47.55%, 47.85%, 16.31%, and 47.55% higher than those under W0N0 treatment, respectively. Appropriate water and nitrogen application can obtain higher WUE and NUE of wolfberry. Results of PCA showed that the yield, WUE, and NUE of wolfberry with mild water deficit (65%–75% θ_f) combined with nitrogen application amount (300 kg/hm^2) was higher, which was an appropriate water and nitrogen control mode for the production of wolfberry in the irrigation area of the Yellow River in the Gansu Province and similar planting areas.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

Conceptualization: GAO Yalin, QI Guangping, MA Yanlin; Methodology: GAO Yalin, MA Yanlin; Formal analysis: GAO Yalin, QI Guangping; Resources: QI Guangping, MA Yanlin, WANG Jianjun; Writing - original draft preparation, GAO Yalin; Writing - review and editing, YIN Minhua, WANG Chen, TIAN Rongrong, XIAO Feng; Supervision: WANG Jinghai; Project administration, LU Qiang; Funding acquisition, QI Guangping. All authors approved the manuscript.

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